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ZOOLOGY.—*The possibility of control of* Heterodera radicicola *and other plant-injurious nemas by means of predatory nemas, especially by* Mononchus papillatus *Bastian.*[1] G. STEINER and HELEN HEINLY. (Communicated by N. A. COBB.)

I. INTRODUCTION

The investigations, the results of which are described in this paper, were carried on from December, 1921 to the end of May, 1922, in the Osborn Zoological Laboratory of Yale University, in collaboration with the U. S. Department of Agriculture. The work was outlined by Dr. N. A. Cobb of the Bureau of Plant Industry. As the investigations were of a novel nature, numerous methods for the rearing of nemas had to be worked out. A large quantity of soil-material was collected and washed by a combination of the sieve method described in an earlier paper by Cobb (7) and the well known gravity-method. The daily control of the cultures, transferring the predatory nemas to fresh conditions, keeping records of the victims and adding a fresh supply of food requires much time and patience. *Heterodera radicicola* material was kindly sent to us by the Connecticut Agricultural Station and from the Plant Introduction Garden of the Department of Agriculture, Brooksville, Florida. We wish here to express our appreciation for this assistance. We feel indebted to the authorities of Yale University, particularly the Osborn Zoological Department, for their permission to carry on our work and for their cordial cooperation.

II. THE PROBLEM

Numerous methods of control of plant injurious nemas have been described in the past. Although some of these methods are very useful, yet the fact remains that today the damage done by nema-pests is enormous and is still increasing.

In recent years the study of free-living nemas has been greatly increased, and as a result the problems connected with nema-pests

[1] From the Osborn Zoological Laboratory, Yale University, New Haven, Conn., in collaboration with the U. S. Department of Agriculture. Received September 9, 1922.

have acquired a new aspect. It was shown by Cobb (4,5,6), Menzel (13) and other investigators that there are in the soil certain species, and even whole genera, of nemas, which in some cases are, and in others may be, predatory and doubtless at least occasionally, are feeding on other kinds of nemas, and even species known as nema-pests. Cobb recorded a series of such observations (4) and suggested first the possibility of using these predatory nemas as a means of decreasing the number of plant injurious nemas in the soil.

In a more recent paper Dr. Menzel (13) compiled all the recorded facts and observations on food and feeding habits from the literature on free-living nemas. He performed also some experiments with a species of *Mononchus*, corroborating the observations of Cobb. He observed that *Mononchus papillatus*, brought together with *Tylenchus sp.*, *Plectus auriculatus*, *Tripyla media* and *Anguillula aceti*, attacked these forms, and killed them either by sucking out their vitals, or by swallowing them whole.

Cobb therefore advocated an investigation of the relationship between predatory *Mononchus* species and other soil-inhabiting and plant-infesting nemas, especially *Heterodera*. If possible the investigations should show to what extent the above mentioned facts regarding the feeding habits of some mononchs are a true expression of the life habits of these animals. If they prove to be a true expression, methods for the propagating and rearing of *Mononchus* should be studied with the view to applying the results for practical purposes in agriculture, especially for fighting the root-knot nema, *Heterodera radicicola*.

The first thing for us to find out was the life history, food, and feeding habits of the predatory mononchs. We chose for our investigations *Mononchus papillatus* Bastian, a species which appeared to be best fitted, first because it seemed one of the easiest to obtain, being among the commonest of mononchs, and second because many observations have been made on the voracity of this form.

Recent investigators (1–3, 8–12 and 14–18) have reared free-living nemas, but as far as we know, mostly forms which feed on decaying matter, and therefore in most cases easily reared in a small amount of suitable medium on slides or in watchglasses. Berliner and Busch and also Byars were the first who used agar as a culture medium for true soil nemas, although the nemas they experimented on were plant-parasites, namely: *Heterodera schachtii* and *Heterodera radicicola*. They planted seeds of oats, etc. on agar and then nematized it with eggs and larvae of *Heterodera*.

There have been seen no published statements made concerning the culture of predatory soil nemas. Menzel seems to have kept *Mononchus papillatus* specimens for only a short time in a small amount of water, simply in order to perform his experiments on their feeding habits. Therefore, it was first necessary to invent simple and practical methods for rearing mononchs.

III. METHODS OF CULTURE OF MONONCHUS PAPILLATUS BASTIAN

The greatest difficulty in rearing minute soil organisms, especially soil nemas, is to devise a culture medium permitting at any time the study and inspection of the reared animals and offering as far as possible natural conditions. Soil, however, even in small quantities, is not transparent. If then, the organism to be cultured is to be continuously observed, ordinary soil cannot be used as a culture medium.

A. Culture in a drop of water on a concave slide.—The first attempt to rear the mononchs was in a small drop of water on a concave slide. *Mononchus papillatus* specimens at different stages of growth were isolated in water-drop cultures, and *Heterodera* larvae, different species of *Rhabditis* or *Anguillula aceti*, added as food. By this method it was possible to keep mononchs alive for from one to three weeks, if the water was changed daily. In cultures of this sort the mononchs seemed to remain in very good condition for several days; they were easy to observe and study, and it was not difficult to get a record of the number of nemas eaten or destroyed by them. However, after a short time had elapsed, all animals reared in pure water cultures became sluggish and finally died.

B. Culture on concave slides with soil and water.—After a number of failures with the foregoing method, we tried to get more natural conditions by adding small quantities of soil to the drop of water on the concave slide. It was seen immediately that the nemas under such conditions grew better, and could be kept alive for a much longer time. After some experience, we were able to rear the mononchs by this method from the first larval stage up to the adult stage and even to the point of producing eggs. The following points should be kept in view.

1. In order to render the observations comparatively easy, only a small quantity of soil should be used. A larger amount of soil furnishes a much better medium for the nemas, but the investigator will have difficulty in finding and controlling them. By trials, any investigator can find the best amount of soil.

2. The nature of the soil is not of great importance. Our experi-

ments showed that sandy soil as well as other kinds may be used.

3. It should be seen to that the soil be free from decaying matter.

4. If possible the water, and even the soil, should be changed daily. *Mononchus papillatus* seems to be very susceptible to any pollution, especially bacterial (*B. subtilis* typ.).

It seems very astonishing at first that the simple addition of a small amount of soil should be of such great importance to the growth and life of these nemas. It was found that soil was absolutely essential for the keeping alive of newly hatched larvae and for rearing them.

C. The agar-culture method.—In 1914 Berliner and Busch described a method for rearing *Heterodera schachtii* Schmidt on agar plates. Agar was carefully washed, sterilized and poured into petri dishes. Sterilized seeds of oats, turnips, beets and different varieties of vetch and clover were planted on the agar and eggs or larvae of *Heterodera schachtii* added. This seemed to be a very simple and useful method also for our purpose, it being only necessary to add the mononchs to get a complete food cycle,—*Heterodera* feeding on the roots of the growing plant and *Mononchus* feeding on *Heterodera*. Byars in 1914 also described a similar method for rearing *Heterodera radicicola*. Had good results been obtained, it would have shown very beautifully the relationship between these two forms of soil nemas; but difficulties soon arose. In the first place *Heterodera radicicola*, the form used for infecting the roots, did not attack them (oats, cucumbers) and up to the present time our experiments along this line have not been satisfactory. The mononchs were already feeding on the *Heterodera* and seemed to thrive in the agar, moving constantly around, even laying a number of eggs. So we added more food for them, consisting of *Rhabditis pellio*, *Rh. elegans*, *Anguillula aceti*, and *Heterodera radicicola*. By this time the bacterial infection of the plate grew so heavy that the mononchs died. Young larvae already hatched from the eggs also died, even in freshly prepared and non-infected agar. Thus it seems to us that the agar method may be used only for larvae a week or more old, and adults, and then the agar should be changed every eight to fourteen days.

A further inconvenience consisted in the size of the plate, since on even the smallest plates it was quite difficult to find the nemas and eggs again, because of the rapid wandering of the former through the agar and the minuteness of the latter.

The second method, that of using concave slides with a small quantity of soil in a drop of water, seemed best fitted for our purpose,

especially in the matter of keeping records of the rate of growth and the number of animals destroyed.

D. The tube-culture method.—Desiring to control the results of our slide cultures, we started, after several other experiments, with cultures in a larger amount of soil in small glass tubes, which were placed in holes bored in a small block of wood, as shown in Fig. 1. A small

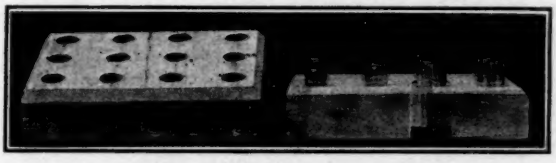

Fig. 1. Wooden block and glass-tubes with soil contents illustrating the tube-culture method of rearing mononchs.

amount of sterilized soil was placed in each of these tubes, moisture added, as well as the mononch to be reared, and a daily supply of *Rhabditis*, *Anguillula* or *Heterodera* as food. The tubes were kept in a moist chamber. If a large amount of soil was used it was not necessary to renovate the tubes for several weeks, but if a smaller amount was used, a weekly change was required. The chief aim was to secure conditions for the mononchs as near as possible to those found in the soil of fields.

Under these conditions it was possible to rear *Mononchus papillatus* in a shorter time than on concave slides. A larger number of eggs was also produced. If a sufficient amount of food was added, numerous mononchs could be reared together in the same tube, without their attacking each other.

The best method for inspecting a tube was to pour the contents into one or more large watchglasses and then dilute with a large quantity of water. If this was not done, eggs and larval forms easily escaped notice.

E. Remarks on flower-pot and field experiments.—Finally a series of host-plant experiments were also performed. Flower pots and soil were sterilized in an autoclave, and sterilized seeds of oats, tomato and cucumber, planted. The seeds were sterilized according to the presoak method, a one-tenth per cent solution of commercial formalin being used. After the first roots were formed and the growth began, the plants were infected with a certain number of *Heterodera radicicola* and also *Mononchus papillatus* added at the same time. Unfortunately

we worked with too small a supply of animals, and could not get noticeable results within the short time available. The roots were attacked by *Heterodera* but the effect of the *Mononchus* was not determined. With further experiments along this line, it should be quite possible to observe results from the external appearance of the host plants.

IV. THE LIFE-HISTORY OF MONONCHUS PAPILLATUS BASTIAN

Comparatively little is accurately known about the life-history of free-living nematodes. About the only forms that have been studied are those living on decaying matter, such as species of *Rhabditis*, *Cephalobus*, *Plectus* and *Diplogaster*. Most of these genera have a very rapid development and are in this way somewhat adapted to the ephemeras of dead and decaying bodies that serve as food. From a study of these forms it would be impossible to generalize and state that all soil or free-living nematodes have a rapid development and short life. The results we obtained in rearing *Mononchus papillatus* showed that the life of this form may be of considerable length, and the same thing may be true of many of the other soil nematodes.

In order to judge the value of *M. papillatus* for fighting other nematodes it is necessary to have a complete knowledge of its life history, the time required to reach the adult stage, the number of eggs produced and the length of life after the cessation of egg production.

Nematodes live in the soil as members of an association of living forms, among which there exist interrelationships. It is important to know what effect *Mononchus* as a predatory form may have on the associated nematode population of the soil, and especially its influence on the diminution of forms causing root-knot and similar diseases; if the monoch has a high rate of propagation, and lives for a long time, it has naturally a more rapid and beneficial effect.

A. The hermaphroditism of Mononchus papillatus.—Hermaphroditism in numerous species of the genus *Mononchus* was first observed by Cobb. Males have been described in very few species, and seem to be extremely rare in most of them. A gradation exists in this genus similar to that found in the genera *Rhabditis* and *Diplogaster*, where we find some species in which there is an equal number of males and females, some in which from ten per cent to forty per cent or more are males, some species in which not even one per cent are to be found and still others in which males seem never to occur.

Conditions similar to these are found in the genus *Mononchus:* the Antarctic *M. gerlachei* seems to produce an equal number of males

and females,—males are rare in *M. obtusus* and in most of the species they have not yet been found.

Hermaphroditism in *Mononchus papillatus* is of some importance in our problem, as every individual is able to reproduce, not being dependent upon the presence of the other sex for propagation. This may prove of great value in using this species as a means of fighting injurious nemas.

B. The egg and the embryonic evolution.—The spermatozoa in *M. papillatus* are very minute, the ovary producing them early, and later on the eggs. Fertilization then takes place. The ovary of the nema is syngonic, and the animal a protandric syngone. (See Cobb 5.) We could not determine the number of sperms produced but it is probably rather restricted, as we observed several cases of old females that formed eggs (at times depositing them also), that were probably not fertilized, as they never developed.

There were never more than two eggs in one uterus at a time, and usually two or three to the individual were observed. It was rather a rare occurrence for the animal to have four eggs, two in each uterus. The eggs were elongated, and appeared smooth shelled in the uterus, but when deposited the surface seemed covered with protuberances, having the appearance of short ridges, the whole surface having a somewhat lace-like effect. (See Fig. 2.)

Fig. 2. Egg of *Mononchus papillatus* showing the protuberances on the egg shell.

If the mononchs lived in an approximately normal condition, an average of two or three eggs was deposited daily. Our slide cultures showed a production of two or less, but the tube cultures gave higher results, and it may be that under natural conditions in the soil the egg production is still higher.

In the slide cultures, the maximum number of eggs deposited by one female was twenty-eight in twelve days, the animal then ceasing to produce; but in tubes our records showed eighteen eggs produced in six days, and forty-one larvae hatched from eggs produced by one female in twenty days were also found. The exact number of eggs produced may have exceeded this, as even with the most careful inspection, eggs and larvae may escape notice. Experiments along this line are still being carried on and higher figures may be obtained.

The number of eggs laid by one *M. papillatus* seems very small when compared with that of some other free-living species, as, for instance, species of *Rhabditis*, in which within a few days eight hundred or more

eggs are produced. The maximum number obtained from one *Mononchus* was forty-one, the minimum number, twenty eggs.

The eggs required from six to seven days before hatching. This also is rather a long period compared with *Rhabditis*, *Diplogaster* and some other known forms. The mononch eggs are unicellular when deposited; the first division takes place about twenty-four hours later.

C. The development of the larva of Mononchus papillatus.—The newly hatched larva is very active but not hardy, and the presence of soil seems very essential to its life. The body is quite transparent. As far as our experiments show, during the first two or three days the larvae attacked no nemas; but on the fourth day, and even on the third day, the predatory instinct was developed and they began to feed on other nemas. As soon as this occurred the intestine began to darken and in a short time became opaque.

The time of larval development up to the period of sexual maturity was somewhat varied. Slide cultures showed the average time for larval development (from the end of embryonic life in the egg shell to sexual maturity) to be from six to seven weeks. But tube cultures showed the minimum time to be only four and one-half weeks. The temperature of these cultures was the same, and the difference in results must be due to the more natural environment found in the tubes. Therefore we are inclined to adopt the minimum time as the time required for development, and to judge from this the influence of this species upon the nematode population of the soil.

Unfortunately, up to the present, we have not been able to get exact data as to the time and number of the moultings. We observed three moults, two in the earlier stages of growth and one just before arrival at sexual maturity. In a study of the life history of one specimen we observed a moult at eighteen days and another at forty-four days after hatching, the latter being the moult just before sexual maturity. Judging from the size of the nema at which other moultings were observed, there must be a moult between eighteen and forty-four days. There may be a moult soon after hatching, but we never observed it. The skin is shed as a whole. There were no observations of the act of moulting, but, judging from the cast, the animal leaves the skin through an opening in the ventral side near the cardia. The mouth-capsule and wall of the rectum were found attached to the cast; the lining of the oesophagus was not observed and may be cast off separately. The animal became rather sluggish and inactive just before each moult.

D. The adult and senile Mononchus papillatus.—Soon after moulting the last time, the mononch began to produce eggs. At this age, the nema was extremely voracious. The number of eggs produced seemed to be in direct proportion to the number of destroyed and devoured nemas.

The maximum duration of egg production observed was twenty days, but further experiments may probably show a longer period.

The period after the cessation of egg production was called the senile stage. It seemed very remarkable that this senile stage was of such long duration as compared with the length of the other periods. In one case the senile period lasted ten weeks, while the combined length of the other periods was only eight weeks. In many hermaphroditic nematodes there are long senile stages. The senior author observed in cultures of the hermaphroditic *Rhabditis elegans* Maupas, the length of the different life periods to be as follows:

April 20—newly hatched larva,—(larval stage lasted two days or less).

April 24—first production of eggs,—(egg production stage lasted two to three days).

April 26—production of eggs stopped,—(senile stage lasted thirteen days).

May 9—death.

Of a life period of eighteen to nineteen days, thirteen were of the senile stage, that is, the period after the last fertilized egg was laid. The senile stage was therefore two or three times longer than the other life periods taken together. The protandric syngonism (Cobb 5) of these forms is probably the cause of this. If spermatozoa are essential and only a certain number of spermatozoa are produced, then when this supply is exhausted, if the gonads are unable to produce more sperms, all subsequent ova will fail of further development, and senility begins.

The same thing seems to happen in *Mononchus papillatus*. The comparatively enormous length of the senile stage may be the result of the inability of the syngone to produce sperm cells a second time. The senile period of ten weeks was observed on a slide culture, and under more favorable conditions it may last very much longer.

In judging the relationship between *Mononchus papillatus* and other soil nematodes the above facts are of some importance, as the amount of food required during the senile stage was very small compared with that devoured during the egg-producing period. Consequently all

senile *M. papillatus* have a decreased efficiency in combating nematode pests.

V. LENGTH OF LIFE OF M. PAPILLATUS

Eighteen weeks was the maximum length of life observed for *M. papillatus* in a slide culture; the tube culture method showed the life period under more normal conditions to be very much longer. Compared with the length of life of other nemas, even excluding forms of *Rhabditis*, *Diplogaster*, *Plectus* spec., etc. that live on decaying matter, this period seems very long, but it may not be exceptional for soil nematodes. At the Zoological Station of Cette (France) the senior author kept adult marine nematodes alive for a comparatively long time, as for example an adult species of *Thoracostoma* for over thirty days. We may therefore conclude that free living nematodes have a much longer life period than is usually supposed.

To a certain degree, this fact may compensate for the restricted number of eggs of *M. papillatus* when considering the effect of this species on the nema population of the soil and the whole community of life therein, as, it means an increase of the efficiency of this species as a destructive factor.

VI. FOOD AND FEEDING-HABITS OF M. PAPILLATUS

In working out our problem, a study of the food and feeding habits was of the greatest interest. From statements made by the investigators already mentioned, it was known that *M. papillatus* fed on other nematodes. It was necessary to discover whether the carnivorous habit lasted throughout its life or for only a certain period. To obtain these facts was of great importance as they determined largely whether *M. papillatus* can become a really efficient agent in combating nematode pests. Our experiments along this line confirmed fully what Cobb in several papers stated to be true. In our cultures, *M. papillatus* lived exclusively on other nemas, also appearing at times to devour small soil particles. The consumption of soil grains seemed to be of chief importance in the first larval stage of the animal, and we observed likewise in the intestine of adults soil particles of unknown origin. The important influence of the presence of soil on our cultures seemed also to confirm these observations.

The larval *M. papillatus*, just hatched from the egg, was very active. Its intestine at first was transparent; the cells of the thin wall at this stage were easily seen, and their nuclei as well. But in a suitable medium, a few days were sufficient to transform this colorless intestine

into a brownish, or even apparently black organ. To this end the presence of soil was absolutely necessary. In water, but with plenty of food (other small larval nematodes) the mononch larvae remained transparent and died in a short time. We were not able to determine exactly what kind of food the newly hatched and very young larvae ate. They moved through the soil, sucking here and there on soil fragments, and particles of an organic nature were probably consumed. But the mononch larvae soon began to feed on other nematodes. We observed three-day old larvae feeding on *Rhabditis* larvae, and their voracity increased with their age.

Our records showed that the number of nematodes killed daily increased as the mononch developed, beginning with one or two destroyed during the third or fourth day of larval life, and attaining a maximal number of sixty-five. In one case as many as eighty-three *Heterodera radicicola* were killed (either swallowed wholly or partly sucked out) by one mononch in one day. The largest amount of food was consumed at the time the maximal number of eggs was produced, and then it decreased during the senile stage of life. During a life time of about twelve weeks one animal killed 1332 nematodes. We are certain

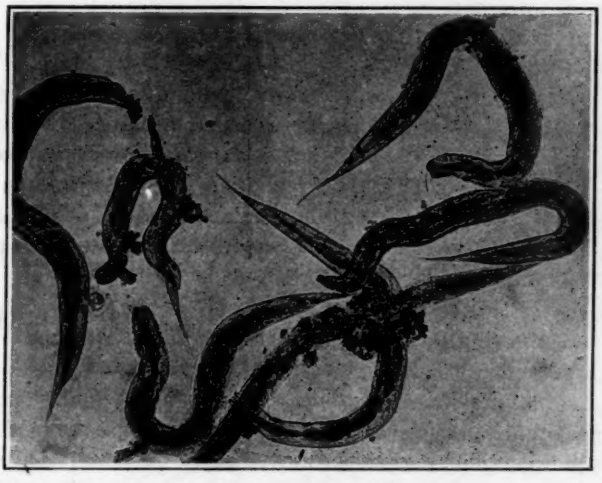

Fig. 3. Photograph of the dead bodies of *Rhabditis elegans* Maupas as they appeared after their slaughter by mononchs. The picture shows well the common method of destruction, that of piercing the cuticle and sucking the contents of the body in the manner of a weasel.

that this number may be very much larger under natural conditions. It was also observed that during the moulting periods fewer nematodes were killed and for a period of one or two days none at all, with a decided increase after the moult.

When in a resting position, the mononch lay on its side, and never on either its back or "stomach." The body was more or less curved ventrally,—the tail end often bent in the form of a spiral. At times the whole body was spirally curved. Agar was the best medium for the study of the movements. It was seen that locomotion was accomplished by bending in the dorso-ventral plane of the body, this plane being placed mostly in a horizontal position so that the dorsal and ventral sides were seen in profile. *Mononchus papillatus* changed continually from a moving to a resting state, moving backward as well as forward.

In the water-drop cultures with a small amount of soil, the mononchs were found only very exceptionally outside the soil area. There was a striking difference in the behavior of *Mononchus papillatus* and the *Rhabdites* added as food. The mononchs seemed to have a more highly developed stereotropism, as they remained well hidden in the soil, while the *Rhabdites* were more or less concentrated on the edge of the water drop outside the area occupied by the particles of soil.

The sense of touch seemed to be the only sense *M. papillatus* used in hunting about for food. It is known that in nematodes three different kinds of sense-organs may be developed.

1 *Organs for the sense of touch.*—These are no doubt among the chief sense organs of the group and show a high degree of perfection in stereotropic reactions and reflex movements.

2 *Chemical or chemico-physical sense organs,*—those known as lateral organs or "amphids." In *M. papillatus* the amphids are rather small and inconspicuous.

3 *Organs for the perception of light.*—These are found in many free-living nematodes, but are absent in mononchs as well as in most soil inhabiting forms.

Judging from the behavior of *M. papillatus*, the organs of touch were probably the only ones used while hunting for food. The nema moved through the medium, continually searching its surroundings by moving the head end in all possible directions. The head end contains the chief organs of touch, as well as those for chemical perception. After long observations, we came to the conclusion that *M. papillatus* was not able to find its prey at long distance, and the use

of the sense organs of touch seemed to be its chief method of locating food. We could therefore confirm statements made by Menzel about this matter.

As soon as the head end of a mononch came into contact with its prey, it grasped it tightly. By a sucking movement, probably of the oesophageal muscles, the head was fixed to the prey. The nematode caught by the mononch naturally made most violent efforts to get free, and often the mononch was shaken and dragged along. But it held on tightly and soon its tooth and other mouth parts began to work. By a sucking movement of the oesophagus and mouth cavity, the mononch became more firmly fixed to its prey, when the tooth located on the dorsal wall was protruded and produced an opening in the skin of the victim. Then a stronger (or further) sucking action was exerted by the mononch and the tooth came back to its normal position. The whole oesophagus came often into action and moved forward and backward with the intestine, the whole body going through the same movements; first the body fluid of the prey was sucked out, and as this happened its body became shrunken. Very often the mononch was satisfied with this, and set free its prey, which died shortly afterwards. Specimens of *Rhabditis elegans* killed in this way are pictured in Fig. 3. *M. papillatus* rarely devoured an entire animal when feeding on large forms, except when exceedingly hungry. No one part of the victim's body seemed to be preferable for attack, the prey being seized at random. Fig. 4 shows an *Anguillula aceti* attacked near the cardia, and

Fig. 4. Photograph of a mononch attacking an *Anguillula aceti* near the cardia. Photograph taken from an agar-plate.

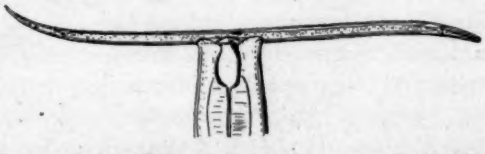

Fig. 5. Sketch of the head-end of a mononch attacking a larval *Rhabditis*. Notice the wide opened lips and the protruded tooth.

Fig. 5–9 in other situations. Larval mononchs were only able to attack and destroy the prey in the way described, but even a small mononch will attack prey of much larger size. Large mononchs often swallow smaller nemas whole; this will happen, if the prey is seized at one end of the body, the animal being then sometimes swallowed without being killed or even injured by the mouth organs of the mononch. In our slide cultures this phenomenon was twice observed. A large adult mononch swallowed a rather large larva of *Rhabditis elegans*. In this species of *Rhabditis* the larvae are very slender and more resistant than other species. The larva seemed to have passed through the mouth and oesophagus of the mononch without harm, probably in the way sketched in Fig. 10. We first noticed that the mononch, very active the day before, appeared nearly motionless. To our great astonishment we noticed the larva of the *Rhabditis* moving throughout the intestine as if searching everywhere for an exit. The larva moved in every possible direction, the mononch becoming less active all the time. We were very interested in knowing the outcome of this case. The digestive fluid in the intestine of the mononch did not seem to harm the larva. The same evening, the mononch did not react to outside stimuli and appeared quite motionless. During the night the *Rhabditis* found a way to freedom through the vulva opening, and in the morning the mononch was dead. The same thing happened shortly afterward with another very active mononch.

Fig. 6. Sketch of the head-end of a mononch seizing an adult *Rhabditis* by the neck. The skin of the prey is already opened, and the mononch is sucking the contents; notice that the dorsal tooth during this sucking action is in its normal position and not protruded. The prey was afterwards sucked out as shown in Fig. 9.

Fig. 7. Sketch of a *Rhabditis* seized near the middle of the body and partly sucked. See Fig. 8 for later stage.

From a scientific point of view these incidents are of interest, as they show that this species of *Rhabditis* is very resistant to mononch

digestive fluids, and they also throw light on the way the mononch swallows, and the action of the dorsal tooth of the mouth cavity. Incidents of the kind just described seem only possible if the tooth, during the act of swallowing, is protruded to such a degree that its point cannot harm the prey. Very probably, the latter was caught by the tail-end, and in the act of seizing, the tooth was protruded and the tail glided into the mouth cavity. In most cases the prey is seized at some other part of the body as Figs. 7–8 show. In this case not only the body fluid is sucked out, but also a large part of the intestine. Fig. 8 shows the prey after release, the skin remaining.

Fig. 8. The *Rhabditis* refered to in Fig. 7 as it was released after having been partly sucked. Notice that the skin of the emptied part remained.

The chief organ of the mononch functioning during the act of swallowing was undoubtedly the oesophagus. The contractions of its radial muscles were vigorous; they began at the mouth cavity and ran down the oesophagus so quickly that at first they seemed instantaneous. But more careful observations showed that each contraction first began around and behind the mouth and then ran back to the intestine. This contraction of the oesophageal radial muscles was accompanied by a shortening of the entire organ, and the whole intestine during the act of swallowing moved back and forth. As soon as the radial muscles contracted, the oesophagus apparently shortened and the forward movement of the cardiac region was easily seen. When contraction ceased; the oesophagus assumed its normal shape and the cardiac region and intestine moved backwards.

Fig. 9. Sketch of a *Rhabditis* sucked out completely at one end, notice the remaining skin.

The mouth cavity did not serve as a place for macerating food, but more or less as a suction capsule. Together with the lips and the attached muscles, it may seize and retain the prey, but our knowledge of the whole apparatus was not sufficient to completely understand its function. The dorsal tooth was certainly protruded as long as the whole cavity was used in retaining the prey.

Some of the digestive fluids are probably formed and secreted by small glandular cells among the radial muscles of the oesophagus. These glands were first described by Cobb. Their secretions may not act in the oesophagus itself, as the food did not stay there. Digestion began and ended in the intestine. The oesophageal glands were probably compressed every time the animal swallowed by the shortening and pressure of the radial muscles, and the digestive fluid thus entered the intestine with the food.

Fig. 10. Sketch of the supposed manner of swallowing the *Rhabditis* larva which was found living and moving in the intestine of a mononch, killing it afterwards by breaking through the intestine and body wall. *dnt.* —tooth as it is supposed to have acted during the attack on the larva.

VII. MONONCHUS PAPILLATUS AS A FACTOR IN FIGHTING NEMATODE PESTS

The foregoing experimental results show conclusively the predatory nature of *Mononchus papillatus*. From our experiments, and from observations made by other investigators, all kinds of nematode species, and Rotifers, Naididae (small Oligochaetes), etc. are taken as food. *Heterodera radicicola* is acceptable in large numbers, and we were able to rear *M. papillatus* by feeding this form of plant-injurious nema exclusively. The same thing happens in the soil and there is no doubt but that mononchs live there in about the same way as in our cultures. Undoubtedly this predatory nema when present kills *Heterodera* and other injurious forms in soil planted with crops. It is therefore extremely useful and its propagation should be encouraged. Under some conditions this form perhaps completely controls *Heterodera* and similar plant-injurious species. Why should this not become the case in our infested fields? Further investigations should be started along these lines.

Once in the roots, *Heterodera* and other such root-parasites are probably protected against the predatory mononchs, but when moving freely in the soil during larval life, they may be destroyed in large numbers and we are convinced that under favorable conditions *Mononchus* is able partially, perhaps completely to control some of these destructive forms. In order to advance any further along this line, it is absolutely necessary to have a knowledge of the nematode population of different kinds of soil, of the relationships that exist

between these forms and other members of the community of living forms that exist there, and of their relationships to other nematode species.

VIII. THE SOIL, ITS LIFE AND THE NUMBER OF NEMATODES, ESPECIALLY OF PREDATORY FORMS THEREIN

Our knowledge of life in the soil, especially of microscopic life there, is still very restricted. We know very little about the relationships that exist between the living components of the soil, and often we do not even know these components. Certainly soil fertility does not depend entirely upon its chemical, colloidal and physical nature, but also and probably largely, upon the animal and plant life therein. The importance of soil bacteria is already known and there exists a close relationship between these bacteria and the nematode population, as a large number of nemas are bacteria consumers. Investigations carried on throughout more recent years have definitely proved that the soil contains an enormous number of nematodes, and that, next to bacteria, they form probably the largest constituent element in subterranean life. Numerous new species have been described and all investigators agree that there is an enormous number still undescribed. It will require the collaboration of many research workers to describe all these forms. A nema species may furnish millions of individuals to every acre of our fields. No exact records regarding the number have been published so far. Dr. Cobb kindly placed at our disposal some of his tables from field surveys. The results of the surveys shown below are of great interest.

	Minimum number of nematodes per acre, top six inches (15.2 cm.)
From Missouri corn field	648,000,000
From North Carolina field	242,400,000
From New Jersey field	129,600,000
From Rhode Island field	610,800,000
From New Hampshire field	99,600,000
From Minnesota field	121,200,000
From Vermont field	580,000,000
From Kansas field	278,400,000

These tables give only the minimum numbers and are figures taken from a study of only the top six inches (15 cm.). A maximum penetration of some nema species has been observed to be as much as (7–8 m.) 25 feet. These unpublished observations were made by Dr. Cobb on citrus trees in California and on alfalfa roots in New South Wales.

Usually soil nematodes go as deep as the roots of plants can penetrate and therefore the depth figures recorded in publications are too small.

Until recently nemas have been almost completely disregarded by investigators of soil economics, and especially by research workers in soil fertility.

If we wish to know what part nematodes play in the economics of the soil, one of the first questions to be answered is what is the nature of their food. There have been no very comprehensive studies made of this question up to the present, and our knowledge is based mostly on only occasional observations. The results of these show that some forms such as *Rhabditis*, *Cephalobus*, etc. feed on decaying matter, that is on the contained bacteria or bacterial products; others, such as *Tylenchus*, *Heterodera*, *Aphelenchus*, etc. feed on plant tissues or fluids; others such as *Dorylaimus*, etc. feed probably on both plants and animals; and still others such as *Mononchus papillatus*, described in this paper, are apparently exclusively predatory. So that not only the problem of combating nematode pests in soil by the use of their natural enemies will be attacked by investigations along this line, but also the problem of soil fertility itself.

Today we know that soil nematodes play a very important role,

1. As consumers and destroyers of our crops;—often destroying whole fields, but much oftener decreasing their yield in a less perceptible degree.

2. As consumers of, and as important workers in the distribution of the bacterial flora of the soil. Their activity is certainly not restricted to denitrifying bacteria, but extends to nitrifying forms as well. A closer study may show relations of very great importance. They carry bacteria and fungus spores everywhere. Wounds on roots or other parts of plants may very often be infected by bacterial and fungus diseases carried by these nematodes. (See Metcalf 15.)

3. As having relationships with fungi upon which some species are known to feed. According to Zopf fungi on the other hand may use nematodes as prey.

4. As consumers of protozoa. It is known that certain fresh-water nemas feed specially on protozoa, sometimes apparently on a single species. There is a probability that some soil species may feed in a similar way, and further investigations may enable us to combat plant injurious protozoa by the use of these nemas.

5. As furnishing a control for plant-injurious nemas. This refers to the work of predatory nemas, such as *Mononchus papillatus*, whose

feeding habits have been described in this paper. There are other species of nemas with a similar significance. Here is a great field for study. Investigations should also be carried on to show more definitely the relations of these predatory forms to rotifers, oligochaetes and other soil animals.

6. As a factor in the humification of the soil, in transformation and removal of all kinds of organic matter, decaying bodies, etc.

7. As assisting in the aeration of the soil. We know how very important aeration of soil is for the growth of bacteria and all kinds of plants. Quite possibly these millions of nematodes living in the soil are highly useful aerators.

In order to solve the problem of the relation of soil nemas to soil fertility, we require a knowledge of the nema population in the soil itself and we also need to know:

1. The composition of the nema population, the species found and their numeric representation in all kinds of soil,—sandy, humus, swampy, dry-soil, hard clay, etc.

2. The horizontal and vertical distribution of nemas in all kinds of soil and the reasons for this.

3. The seasonal changes of the different species, the number of species and the number of individuals of each species.

4. The influence of habitat on the nematode population including physical, chemical and biological factors found in the habitat. The biological factors include kinds of crops and other forms of vegetation that may be present.

The working out of these problems will surely enable us to conclude why nema pests are absent in some cases, and why in apparently similar conditions they exist in such enormous numbers. A large field is thus opened up for investigations, the results of which will be of great importance to agricultural science.

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